The effect of microcurrent therapy on repair of induced tendon injury in albino rat: possible role of endogenous stem cells

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Received 8 August 2012
Accepted 31 October 2012

The Egyptian Journal of Histology
2013, 36:400-408
37 (1376-2013)

Background
Tendon repair involves a slow repair process, which results in inferior repair of tissue and failure to obtain full active range of motion. Microcurrent therapy (MCT) is a new therapy after arthroplasty. Stem cells with capacity of self-renewal are ideal for tissue engineering.

Aim
The present work aimed at investigating the effect of MCT and the possible role of endogenous stem cells in repair of induced tendon injury in albino rat.

Materials and methods
Twenty-four male albino rats were classified into: Control group, tenotomy group, 5 rats were sacrificed 2 weeks and 5 rats sacrificed 4 weeks following achillis tendon injury (Subgroups IIa and IIb respectively). MCT group of 10 rats subjected to tendon injury followed by MCT, 5 rats sacrificed 2 weeks and 5 rats sacrificed 4 weeks following MCT (Subgroups IIIa and IIIb respectively). Tendon sections were stained with H&E and CD44 and CD105 immunostains. A morphometric study was performed.

Results
Subgroup IIa demonstrated multiple areas of widely separated collagen fibers, infiltrating cells and multiple congested vessels. Subgroup IIb showed multiple areas of disorganized collagen bundles, less infiltrating cells and few congested vessels. MCT subgroup IIIa revealed some areas of parallel collagen bundles, some infiltrating cells and some dilated congested vessels. MCT subgroup IIIb showed multiple areas of parallel collagen bundles, few infiltrating cells and occasional congested vessels. CD44 +ve and CD105 +ve cells were seen among disorganized, parallel collagen fibers and inside blood vessels. A significant increase in the area of regenerated collagen bundles was recorded in MCT subgroup IIIb. The area% of CD44 +ve and CD105 +ve MSCs denoted a significant increase in MCT subgroup IIIa.

Conclusion
MCT activated endogenous bone marrow derived MSCs migration to the injured achillis tendon, which stimulated tendon repair following induced tenotomy.

Keywords:
tendon injury, microcurrent, stem cells

Egypt J Histol 36:400-408

1110-0559

Introduction

Tendon injury is either traumatic or pathological. Surgical treatment of tendon ruptures and lacerations is currently the most common therapeutic modality. Tendon repair involves a slow repair process, which results in inferior repair of tissue and often a failure to obtain full active range of motion [1]. Most injuries are open injuries; less frequent injuries include damage to the functional system of tendon sheath [2]. Grading of tendon injury ranges from stretch to complete rupture [3].

Microcurrent therapy (MCT) is a new therapy for wound healing after arthroplasty [4]. The influence of MCT on the compromised speech functions in children presenting with cerebral palsy was compared with medicomental therapy. The microcurrent promoted positive dynamics of locomotor and cognitive disorders [5].

Tendon is a unique connective tissue with poor self-repair capability. With advances in stem cell biology, tissue engineering is becoming increasingly powerful for tissue regeneration. Stem cells with a capacity of multipotency and self-renewal are an ideal cell source for tissue engineering [6]. The use of mesenchymal stem cells (MSCs) could result in enhanced tendon healing

 DOI: 10.1097/01.EHX.0000428367.71841.b8

and regeneration [7]. MSCs are a therapeutic strategy with anti-inflammatory capability [8].

The present work aimed to determine the effect of MCT and the possible role of endogenous stem cells in repair of induced tendon injury in albino rat.

Materials and methods

The study was carried out at the Animal House of Kasr El- Aini, Faculty of Medicine, Cairo University, according to the guide for the care and use of laboratory animals.

Experimental animals

Twenty-four adult male albino rats weighing 150–200 *g* were housed under good hygienic conditions, fed ad libitum, and allowed free water supply. The animals were classified into three groups, which were kept in separate cages.

Control group (*group I*)

The control group included four rats that were not subjected to injury of the Achilles tendon.

Tenotomy group (*group II*)

This group included 10 rats subjected to Achilles tendon injury. To induce anesthesia, ketamine hydrochloride (Ketalar; Parke Davis, Barcelona, Spain) (35 mg/kg) was injected into the gluteus maximus muscle of the animal. Using aseptic techniques, a 3 cm longitudinal skin incision was made along the leg.

Sharp transection of the tendon was performed using a scalpel about 1 cm apart from calcaneal insertion. The tendon edges were approximated and sutured by 4/0 proline (Ethicon, Ohio-Cincinnati, USA). The fascia and skin were sutured [9]. Betadine was applied to the wound site and rinsing with normal saline was performed at the site of injury daily. The rats were subdivided into two subgroups:

(1) *Subgroup IIa:* five rats sacrificed 2 weeks following the day of injury.
(2) *Subgroup IIb:* five rats sacrificed 4 weeks following the day of injury.

Microcurrent therapy group (*group III*)

This group included 10 rats subjected to Achilles tendon injury in the same way as in the tenotomy group. A microcurrent electric stimulator, model EMSI-4250, Electrostim Medical Services Inc. (Taiwan), was applied 2 days following tenotomy. The active electrode (1.0×1.0 cm) was placed over the tendon injury site, whereas the inactive electrode was placed proximally on the thigh region of the same side, ~3 cm apart. Clip electrodes were used and MCT was applied 3 sessions/week, each for 30 min [10]. The animals were subdivided into two subgroups:

(1) *Subgroup IIIa*: five rats sacrificed 2 weeks following the day of MCT.
(2) *Subgroup IIIb*: five rats sacrificed 4 weeks following the day of MCT.

The animals were sacrificed by a lethal dose of ether. The Achilles tendon was exposed and tendon specimens were placed in 10% formol saline. Five-µm-thick sections were prepared and subjected to the following studies:

(1) *Histological study*: Histological examination was carried out using H&E stain [11].
(2) *Immunohistochemical study* (*CD44 immunostaining*): MSC marker [12] was added as 0.1 ml ready-to-use primary antibody (CD44) rabbit polyclonal antibody (catalogue number IW-PA1021; Immunohistochemistry World, Corporate Court, Ellicott City, USA.), and incubated at room temperature in a moist chamber for 60 min. Tonsil was used as a positive control specimen. Reaction localization is the cell membrane. However, one of the tendon sections was used as a negative control by passing the step of applying the primary antibody.
(3) *Immunohistochemical study* (*CD105 immunostaining*): MSC marker [13] was added as 0.1 ml ready-to-use primary antibody (CD105) goat polyclonal antibody (catalogue number 559286, BD Biosciences, San Jose, California, USA), and incubated at room temperature in a moist chamber for 60 min. Tonsil was used as a positive control specimen. Reaction localization is the cell membrane. However, one of the tendon sections was used as a negative control by passing the step of applying the primary antibody.
(4) *Morphometric study*: Using a Leica Quin 500 (Leica Ltd, Cambridge, UK) computerized image analysis system, the area of regenerated (parallel) collagen bundles was measured in 10 low-power fields in H&E-stained sections using the interactive measurements menu. The area% of CD44 and CD105+ MSCs were determined in 10 high-power fields using the binary mode.

Statistical analysis

Quantitative data were summarized as mean and SD and compared using one-way analysis of variance. *P*-values less than 0.05 were considered statistically significant. Calculations were carried out using SPSS software, New York, USA [14].

Results

Histological results

Sections in the tendon of control rats showed parallel collagen bundles and tendinocytes (Fig. 1). Closer observation indicated tendinocytes with flat nuclei in between parallel collagen bundles (Fig. 2).

Sections in the tendon of rats in tenotomy subgroup IIa (sacrificed 2 weeks following injury) showed localized areas of parallel collagen bundles surrounded by disorganized collagen bundles. Widely separated collagen fibers, infiltrating cells, and congested vessels were evident (Fig. 3). Closer observation indicated multiple spindle-shaped cells and few parallel collagen

fibers. Multiple congested vessels and infiltrating cells were evident (Fig. 4).

Sections in the tendon of rats in tenotomy subgroup IIb (sacrificed 4 weeks following injury) showed occasional areas of parallel collagen bundles and multiple areas of disorganized collagen bundles. However, no disrupted fibers were detected. Some infiltrating cells and few congested vessels were observed (Fig. 5). Parallel fibroblasts and fibrocytes were found in between parallel collagen bundles surrounded by disorganized ones on close observation (Fig. 6).

Sections in the tendon of rats in MCT subgroup IIIa (sacrificed 2 weeks following MCT) showed some areas of parallel collagen bundles, some infiltrating cells, and few congested vessels (Fig. 7). Some areas of parallel collagen bundles containing multiple fibroblasts and fibrocytes in addition to obviously dilated congested vessels were observed (Fig. 8). Other areas of parallel collagen bundles were surrounded by collagen fibers infiltrated by mononuclear cells (Fig. 9).

Sections in the tendon of rats in MCT subgroup IIIb (sacrificed 4 weeks following MCT) showed multiple areas of parallel collagen bundles. Few infiltrating cells and occasional congested vessels were detected (Fig. 10). Close observation indicated wide areas of parallel collagen bundles with few fibroblasts and multiple fibrocytes in between (Fig. 11).

Immunohistochemical results

Sections in the tendon of control rats showed CD44– immunostaining (Fig. 12). In tenotomy subgroup IIa, few branched and spindle-shaped CD44+ cells were observed among disorganized collagen fibers and inside multiple blood vessels (Fig. 13). In tenotomy subgroup IIb, few spindle-shaped positive cells were found among fibroblasts and inside a few blood vessels (Fig. 14). In MCT subgroup IIIa, multiple spindle-shaped CD44+ cells were observed among disorganized, parallel collagen bundles and inside blood vessels (Fig. 15). In MCT subgroup IIIb, some spindle-shaped positive cells were observed around blood vessels present among wide areas of parallel collagen bundles (Fig. 16).

Sections in the tendon of control rats showed CD105– immunostaining. In tenotomy subgroup IIa, few spindle-shaped CD105+ cells were observed among disorganized collagen fibers and around blood vessels (Fig. 17). In tenotomy subgroup IIb, few spindle-shaped positive cells were found among the collagen fibers (Fig. 18). In MCT subgroup IIIa, multiple spindle-shaped CD105+ cells were observed among disorganized, parallel collagen bundles and around blood vessels (Fig. 19). In MCT subgroup IIIb, some spindle-shaped positive cells were observed among wide areas of parallel collagen bundles (Fig. 20).

Morphometric results

A significant increase ($P<0.05$) in the area of regenerated collagen bundles was observed in MCT subgroup IIIa compared with tenotomy subgroups IIa and IIb. In addition, a significant increase ($P<0.05$) was found in subgroup IIIb compared with MCT subgroup IIIa (Table 1).

The area% of CD44 and CD105+ MSCs showed a significant increase ($P<0.05$) in subgroup IIIb compared with subgroups IIa and IIb. In addition, a significant increase ($P<0.05$) was found in subgroup IIIa compared with subgroup IIIb (Table 1).

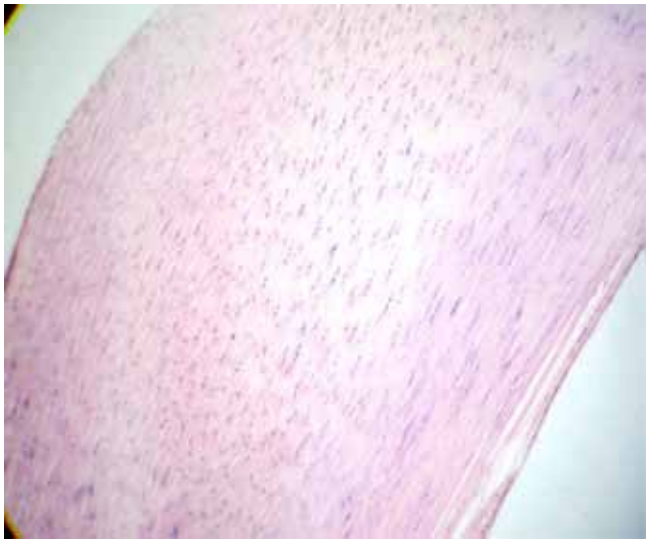

Figure 1. Photomicrograph of a section in the tendon of a control rat showing parallel collagen bundles and tendinocytes.
H&E, × 100.

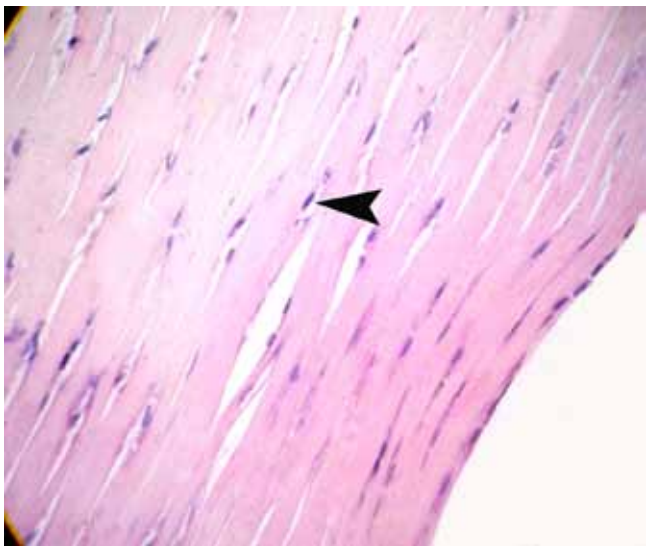

Figure 2. Higher magnification of the previous section showing tendinocytes with flat nuclei (arrow head) in between parallel collagen bundles.
H&E, × 400.

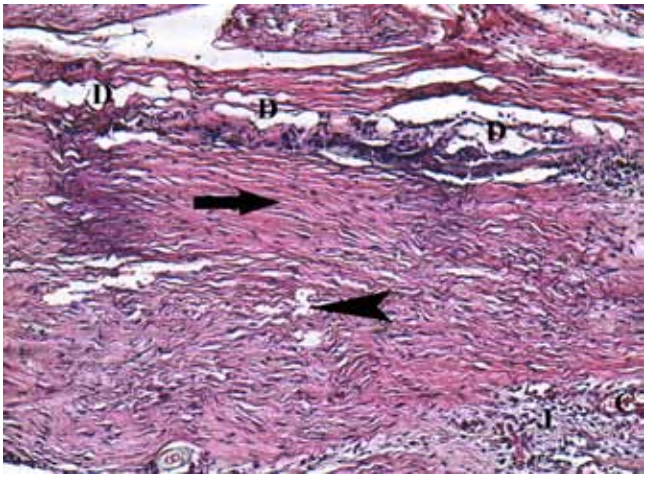

Figure 3. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIa (sacrificed 2 weeks following injury) showing a localized area of parallel collagen bundles (arrow) surrounded by disorganized collagen bundles (arrow head). Note the widely separated collagen fibers (D), infiltrating cells (I), and congested vessels (C).
H&E, × 100.

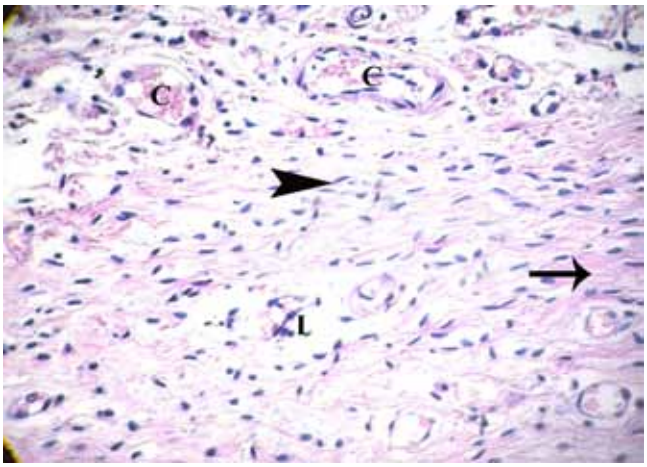

Figure 4. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIa showing multiple spindle-shaped cells (arrow head), multiple congested vessels (C), and infiltrating cells (I). Note few parallel collagen fibers (arrow).
H&E, × 400.

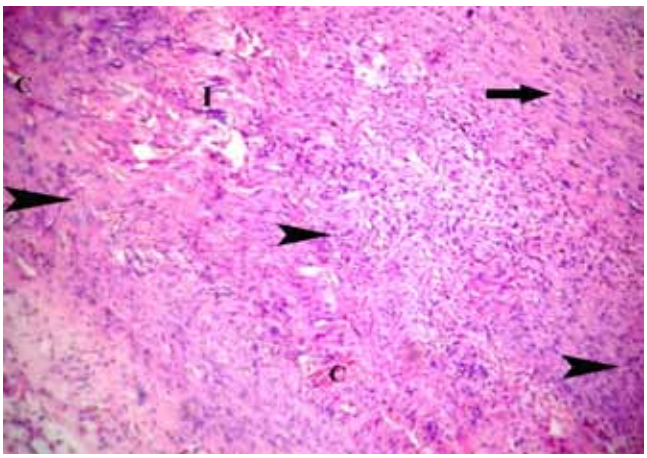

Figure 5. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIb (sacrificed 4 weeks following injury) showing an area of parallel collagen bundles (arrow) and multiple areas of disorganized collagen bundles (arrow heads), some infiltrating cells (I), and few congested vessels (C).
H&E, × 100.

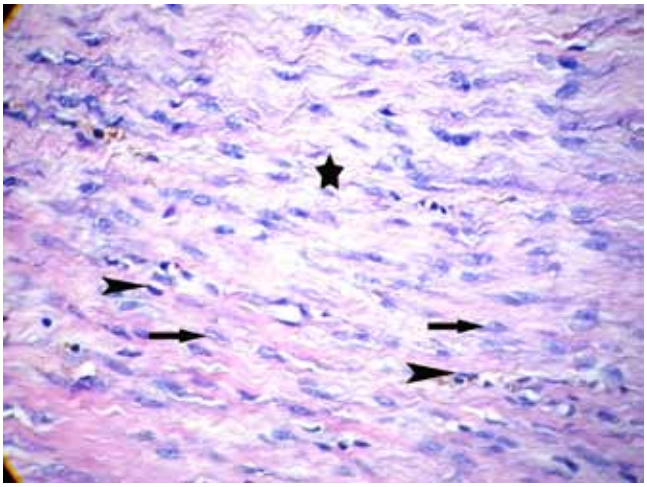

Figure 6. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIb showing an area with parallel fibroblasts (arrows) and fibrocytes (arrow heads) in between parallel collagen bundles. Note disorganized bundles and cells (*).
H&E, × 400.

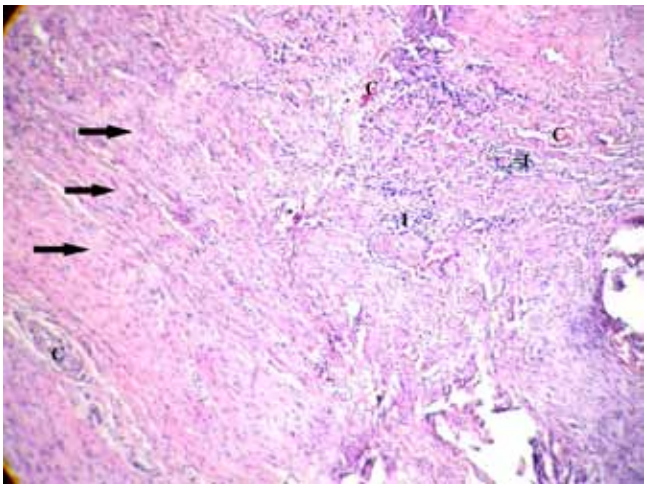

Figure 7. Photomicrograph of a section in the tendon of a rat in microcurrent therapy (MCT) subgroup IIIa (sacrificed 2 weeks following MCT) showing some areas of parallel collagen bundles (arrows), some infiltrating cells (I), and few congested vessels (C).
H&E, × 100.

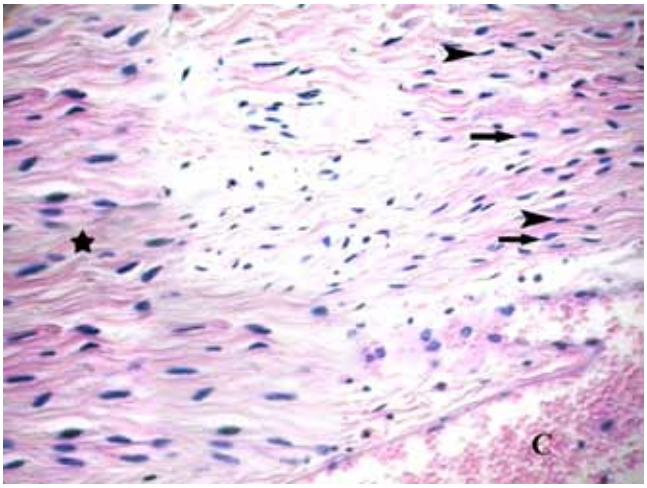

Figure 8. Photomicrograph of a section in the tendon of a rat in microcurrent therapy subgroup IIIa showing some areas of parallel collagen bundles (*) containing multiple fibroblasts (arrows) and fibrocytes (arrow heads). Note obviously dilated congested vessel (C).
H&E, × 400.

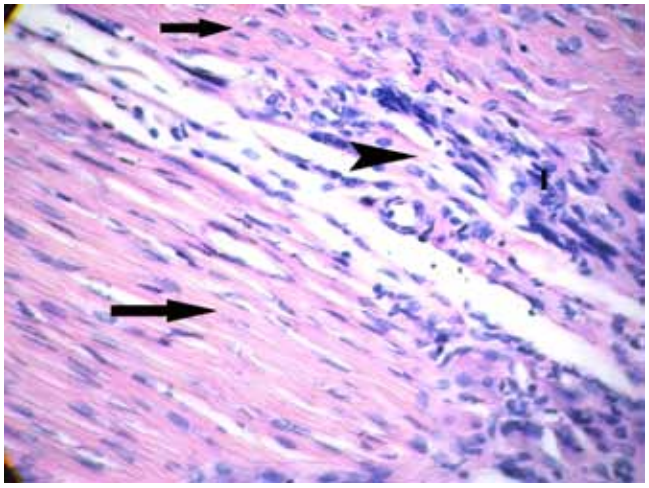

Figure 9. Photomicrograph of a section in the tendon of a rat in microcurrent therapy subgroup IIIa showing some areas of parallel collagen bundles (arrows) surrounding disorganized collagen fibers (arrow head) infiltrated by mononuclear cells (I).

H&E, × 400.

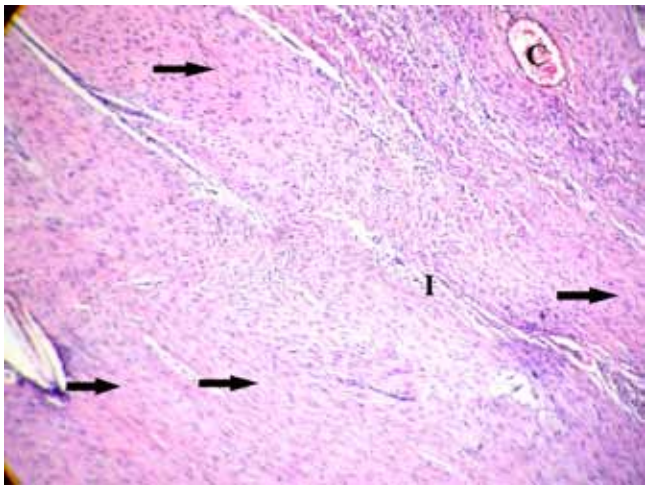

Figure 10. Photomicrograph of a section in the tendon of a rat in microcurrent therapy (MCT) subgroup IIIb (sacrificed 4 weeks following MCT) showing multiple areas of parallel collagen bundles (arrows). Note few infiltrating cells (I) and a congested vessel (C).

H&E, × 100.

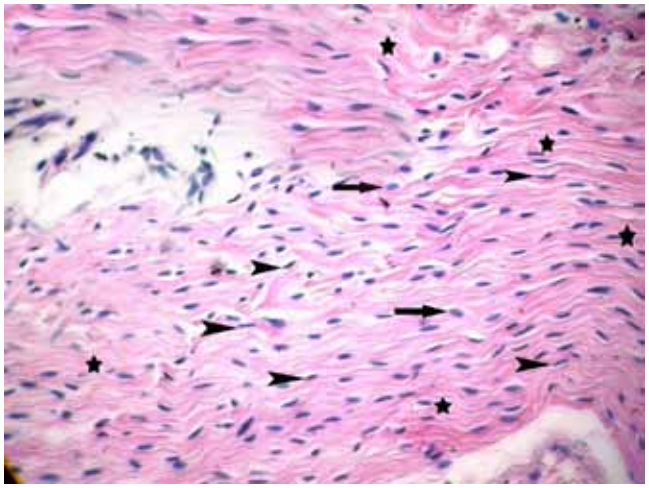

Figure 11. Photomicrograph of a section in the tendon of a rat in microcurrent therapy subgroup IIIb showing a wide area of parallel collagen bundles (*) with few fibroblasts (arrows) and multiple fibrocytes (arrow heads) inbetween.

H&E, × 400.

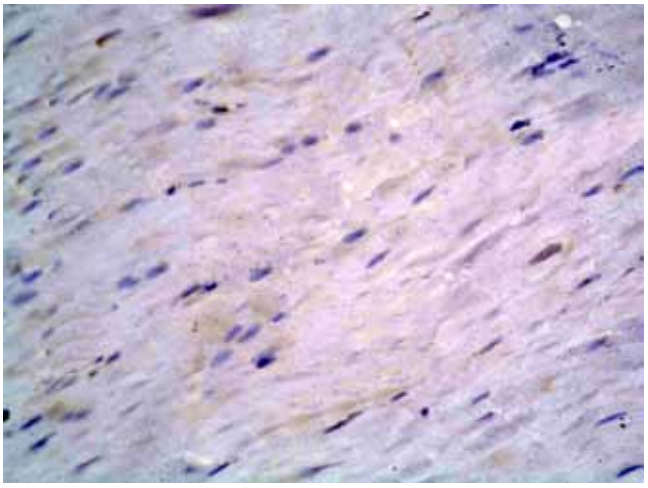

Figure 12. Photomicrograph of a section in the tendon of a control rat showing negative immunostaining.

CD44 immunostaining, × 400.

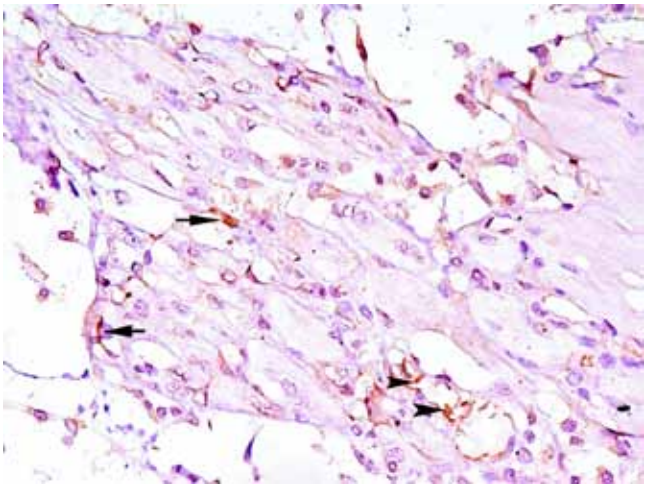

Figure 13. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIa showing some branched and spindle-shaped positive cells among disorganized collagen fibers (arrows) and inside blood vessels (arrow heads).

CD44 immunostaining, × 400.

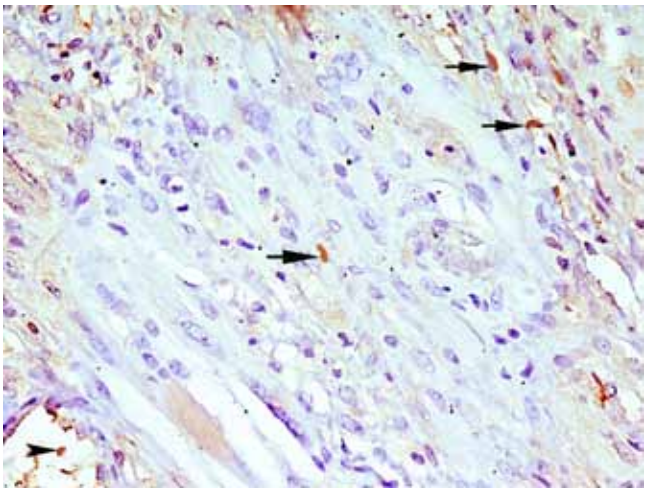

Figure 14. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIb showing few spindle-shaped positive cells among fibroblasts (arrows) and inside a blood vessel (arrow head).

CD44 immunostaining, × 400.

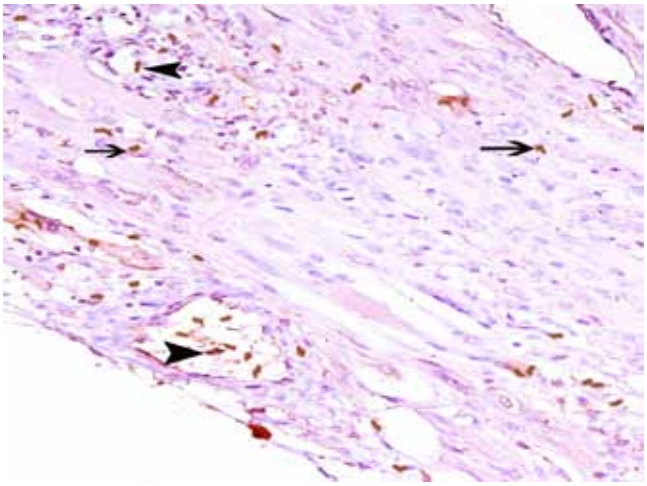

Figure 15. Photomicrograph of a section in the tendon of a rat in microcurrent therapy subgroup IIIa showing multiple spindle-shaped positive cells among disorganized and parallel collagen bundles (arrows) and inside blood vessels (arrow heads).
CD44 immunostaining, × 400.

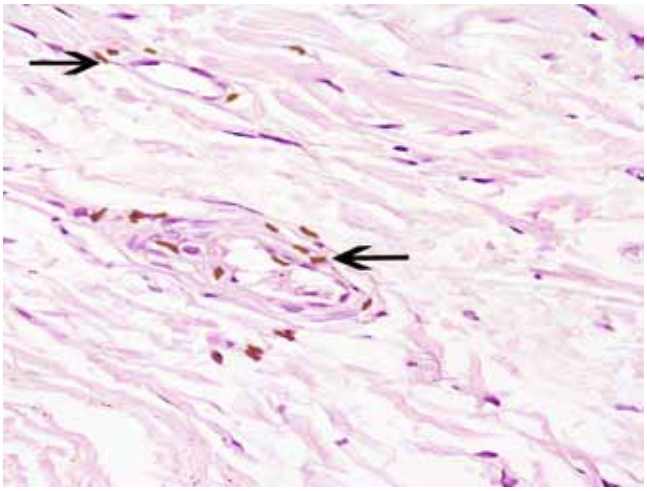

Figure 16. Photomicrograph of a section in the tendon of a rat in microcurrent therapy subgroup IIIb showing some spindle-shaped positive cells around blood vessels present among a wide area of parallel collagen bundles (arrows).
CD44 immunostaining, × 400.

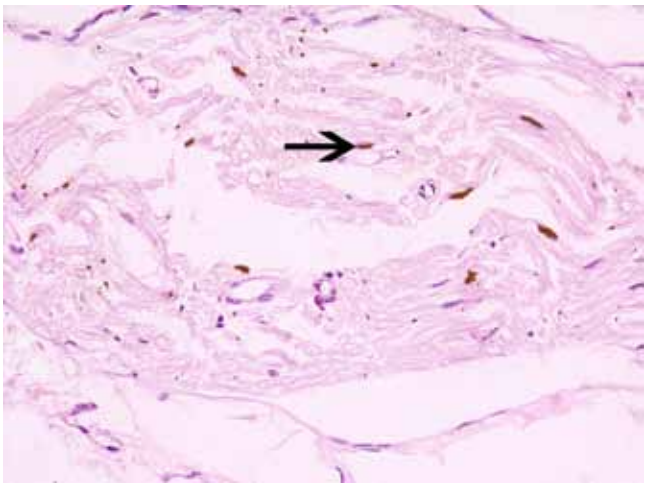

Figure 17. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIa showing few spindle-shaped positive cells among disorganized collagen fibers and around blood vessels (arrow).
CD105 immunostaining, × 400.

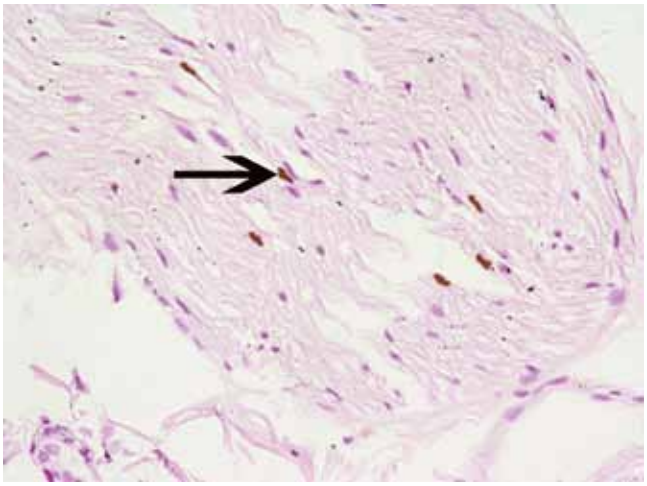

Figure 18. Photomicrograph of a section in the tendon of a rat in tenotomy subgroup IIb showing few spindle-shaped positive cells among collagen fibers (arrow).
CD105 immunostaining, × 400.

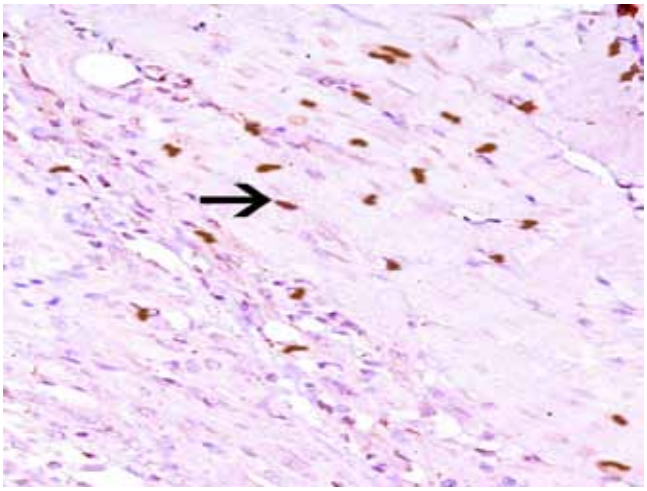

Figure 19. Photomicrograph of a section in the tendon of a rat in MCT subgroup IIIa showing multiple spindle shaped +ve cells among disorganized and parallel collagen bundles (arrow) and around blood vessels.
CD105 immunostaining, × 400.

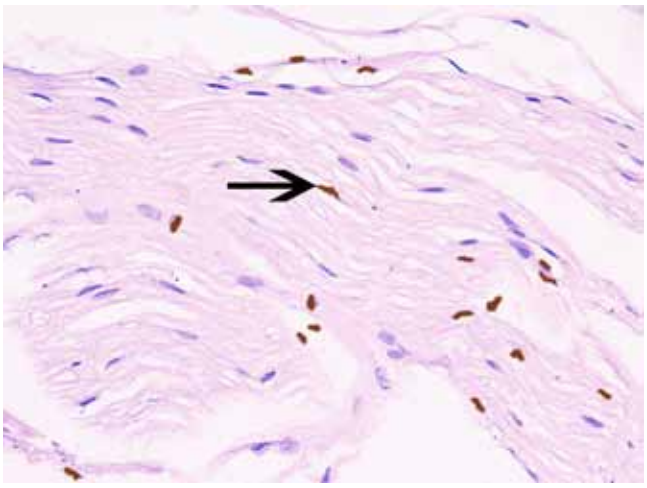

Figure 20. Photomicrograph of a section in the tendon of a rat in microcurrent therapy subgroup IIIb showing some spindle-shaped positive cells among a wide area of parallel collagen bundles (arrow).
CD105 immunostaining × 400.

Table 1. Mean±SD of area of regenerated collagen bundles, area% of CD44+ and CD105+ mesenchymal stem cells

Subgroups	Mean area of regenerated collagen bundles	Mean area% of CD44+ MSCs	Mean area% of CD105+ MSCs
Tenotomy IIa	131.4 ± 8.5	6.2 ± 1.2	5.9 ± 1.0
Tenotomy IIb	167.1 ± 7.9	4.1 ± 0.9	4.2 ± 0.8
MCT IIIa	304.4 ± 11.1*	23.1 ± 2.7*,***	22.5 ± 1.9*,***
MCT IIIb	427.9 ± 13.9*,**	11.7 ± 2.9*	10.8 ± 2.4*

MCT, microcurrent therapy; MSC, mesenchymal stem cell.
*P<0.05 compared with IIa and IIb.
**P<0.05 compared with IIIa.
***P<0.05 compared with IIIb.

Discussion

The current study showed the modulating effect of MCT on induced Achilles tendon injury, which was associated with the existence of MSCs in albino rat. This was evidenced by histological, immunohistochemical, and morphometric studies.

Tenotomy subgroup IIa (sacrificed 2 weeks following injury) showed localized areas of parallel collagen bundles surrounded by disorganized bundles and disrupted collagen fibers. Tenotomy subgroup IIb (sacrificed 2 weeks following injury) showed occasional areas of parallel collagen bundles and multiple areas of disorganized collagen bundles, but no obvious disrupted fibers.

Histological examination showed alignment of collagen fibrils following tendon injury in mice. Thirteen days following tendon transection, granular appearance of dense aligned collagen fibrils developed. The fibers increased in diameter with time [15].

In subgroup IIa, infiltrating cells and multiple congested vessels were evident. Multiple fibroblasts and fibrocytes were intermingled with disorganized collagen, whereas in subgroup IIb, fewer infiltrating cells and few congested vessels were observed. Multiple parallel fibroblasts and fibrocytes were found in between parallel collagen bundles surrounded by disorganized ones.

An inflammatory reaction was observed in the repair areas of transected ends of sutured tendon by the end of second week following suturing of injured tendon. Tendon adhesion and tensile strength increased with time post-injury-suture repair, as did the expression of fibroblast growth factor and collagen type I protein in the injured area [16].

MCT subgroup IIIa (sacrificed 2 weeks following MCT) showed some areas of parallel collagen bundles, some infiltrating cells, and few congested vessels. Multiple parallel fibroblasts and fibrocytes in addition to obviously dilated congested vessels were noted. MCT subgroup IIIb (sacrificed 4 weeks following MCT) showed multiple areas of parallel collagen bundles. Few infiltrating cells and occasional congested vessels were detected. Fibroblasts were few, whereas fibrocytes were more common. Morphological results were confirmed by morphometric results, where a significant increase in the area of regenerated collagen bundles was detected in the MCT group compared with the tenotomy group. In addition, a significant increase was found in subgroup IIIb compared with subgroup IIIa.

The microcurrent skin therapy following hip arthroplasty led to a reduction in the requirements of postoperative analgesics, with an improvement in degrees of wound healing. However, there were several incidences of skin dermatitis [4]. In the case of bone fracture, under the influence of the direct current, the organization of the collagen fibers on several planes was increased because of the action of mechanical forces, whereas in the case of the nonstimulated callus, the arrangement of the collagen fibers was apparently random, with afribrillar areas mixed with areas poor in collagen. The proliferation of fibroblasts was stimulated, facilitating collagen synthesis and angiogenesis [17]. The use of electric stimulation therapy potentially represents a cost-effective treatment in the management of chronic, nonhealing venous leg ulcers [18].

However, low or high doses of microcurrent therapies improved the strength of repair of the Achilles tendon. However, in view of its short treatment time, ultrasound was considered to be more efficient [19].

A 12-week study showed high therapeutic efficiency of cell stimulation with low-intensity electric current on lateral epicondylitis. This could be attributed to upregulation of intracellular transmitters, interleukins, and prostaglandins playing the key role in the regulation of inflammation [20]. A recent study suggested that a low-intensity direct current promotes migration of human dermal fibroblasts to the negative pole. This ensured microcurrent efficacy for pressure ulcer healing [21]. However, limited evidence suggests MCT therapy to be an effective intervention for Achilles tendinopathy [22].

In tenotomy subgroup IIa, few branched and spindle-shaped CD44+ and CD105+ cells were observed among disorganized collagen fibers and inside multiple blood vessels. In tenotomy subgroup IIb, few spindle-shaped positive cells were found among fibroblasts and inside a few blood vessels. MCT subgroup IIIa showed multiple spindle-shaped CD44+ and CD105+ cells among disorganized, parallel collagen bundles and inside blood vessels. In MCT subgroup IIIb, some spindle-shaped positive cells were observed around blood vessels present among wide areas of parallel collagen bundles. Concomitantly, a significant increase in the mean area% of CD44+ and CD105+ cells was observed in the MCT group compared with the tenotomy group. In addition, a

significant increase was found in subgroup IIIa compared with subgroup IIIb, which indicates that stem cell differentiation increases with time.

Collagen fibril diameter and alignment were the measures of stem cell-induced tendon repair [23]. A study was carried out to develop novel stem cell-based therapy and ultimately to achieve more effective repair or regeneration of injured tendons [6]. Advances in biomaterial technology will improve the methodology in tendon regeneration. However, to date, ASCs have been an ideal cell source for tendon engineering [24]. Human and mouse tendons were proved to contain stem cells, referred to as tendon stem or progenitor cells [25].

Recent studies have proved the capability of stem cells to differentiate into tenocytes because stem cells have regeneration potential. The tissue produced is similar to the preinjury state, but the results may vary. The use of adjuncts such as molecular signaling and mechanical stimulation is possible [26]. Stem cells are an attractive option for the augmentation of tendon repairs. Several studies have evaluated the use of MSCs with some success, but profound results have been lacking when these cells are applied untreated. Molecular cues drive MSCs toward tenocyte differentiation [27].

The proliferative capacities of bone marrow-derived MSCs were determined. Their isolation was based on the morphology and expression of CD44 [28]. However, primary MSCs and bone marrow progenitors physiologically lack the expression of CD44 [29].

It could be concluded that MCT activated and increased endogenous bone marrow-derived MSCs migration to the injured Achilles tendon, which stimulated tendon repair following induced tenotomy.

Acknowledgements

Conflicts of interest

There is no conflict of interest to declare.

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الملخص العربى

اثر العلاج بالتيار المتناهى الصغر علي اصلاح الاصابة المحدثة بالوترفي الفار الابيض والدورالمحتمل للخلايا الجذعية الداخلية

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الخلفية: اصلاح الوتر يشمل عملية اصلاح بطيئة مما يؤدى الى اصلاح اقل كفاءة للنسيج و فشل فى الحصول على مدى كامل نشيط من الحركة. العلاج بالتيار المتناهى الصغر علاج جديد لالتئام الجروح بعد تعويض المفاصل. الخلايا الجذعية ذات القدرة المتعددة على التجدد مصدر مثالى لهندسة الانسجة.

الهدف: هدف العمل الحالى الى بحث اثر العلاج بالتيار المتناهى الصغرو الدورالمحتمل للخلايا الجذعية الداخلية فى اصلاح الاصابة المحدثة بالوتر فى الفار الابيض.

الادوات و الطرق: تم تقسيم 24 من ذكور الفئران البيضاء الى مجموعة ضابطة (المجموعة 1) من 4 فئران و مجموعة اصابة الوتر (المجموعة 2) حيث تم تعريض10 فئران لاصابة وتر اخيل وقد تم التضحية بخمس فئران بعد اسبوعين و خمس فئران بعد 4 اسابيع من الاصابة (المجموعتين الفرعيتين 2 ا و 2 ب على التوالى). و مجموعة التيار المتناهي الصغر(المجموعه 3) حيث تم تعريض 10 فئران بعد اصابة الوتر الى الحث بالتيار المتناهي الصغر و تم التضحية بخمس فئران بعد اسبوعين و خمس فئران بعد 4 اسابيع من العلاج (المجموعتين الفرعيتين 3 ا و3 ب على التوالى) ثم تم صباغة قطاعات الوتر بصبغة الهماتوكسلين و الايوسين و الصبغة المناعية سى دى 44 وسي دي 105 كما تم القيام بعمل دراسة كمية قياسية و تحليل احصائى لها.

النتائج: اوضحت المجموعه الفرعيه 2 ا مناطق متعددة من الياف الكولاجين المتباعدة، خلايا متخلله، اوعيه دمويه متعدده محتقنه و خلايا مغزلية الشكل مع وجود القليل من الياف الكولاجين المتوازيه. فى المجموعه الفرعيه 2 ب شوهدت احيانا مناطق ذات حزم كولاجين متوازيه، مناطق متعدده من الحزم الغير منتظمه، خلايا متخلله و اوعيه دمويه محتقنه اقل. المجموعه الفرعيه3 ا اظهرت بعض المناطق بها حزم الكولاجين متوازيه، بعض الخلايا المتخلله و الاوعيه الدمويه المتسعه و المحتقنه بوضوح. المجموعه الفرعيه 3 ب كشفت عن مناطق متعدده من حزم الكولاجين المتوازيه، القليل من الخلايا المتخلله و احيانا اوعيه دمويه محتقنه. شوهدت خلايا السى دى 44 الموجبه متفرعه و مغزليه الشكل على امتداد حزم الكولاجين الغير منتظمه و المتوازيه بالاضافه الى داخل الاوعيه الدمويه. تم تسجيل زياده ذات دلاله احصائيه فى مساحة حزم الكولاجين المتجدده فى المجموعه الفرعيه 3 ب مقارنة بالمجموعات الفرعيه الاخرى. دلت نسبة مساحة خلايا السى دى44 و السي دي 105 الموجبه على زياده ذات دلاله احصائيه فى المجموعه الفرعيه 3 ا مقارنة بالمجموعات الفرعيه الاخرى.

الخلاصه: ادي العلاج بالتيار المتناهى الصغرالي تنشيط و زيادة هجرة الخلايا الجذعيه المزنشميه الداخليه المستمده من النخاع الشوكى الى وتر اخيل المصاب مما استحث اصلاح الوتر بعد الاصابة المحدثة به.